MUTUAL ARRANGEMENT OF HISTONE H1 MOLECULES IN CHROMATIN OF INTACT NUCLEI

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1. Introduction

The structural role of histones H2A, H2B, H3 and H4 in chromatin now seems to be rather well established and involves formation of nucleosome cores (reviewed [1,2]). At the same time, the structural and functional role of histone H1 is still obscure.

The H1 histone molecule consists of three structural domains: the cationic N-terminal part or 'nose' (amino acid residues 1–35), the globular part or 'head' (residues 42–112) and the highly basic structureless C-terminal part or 'tail' (residues 120–213) [3]. The structural domains apparently take part in different histone–histone and histone–DNA interactions [3–5]. Histone H1 molecules in chromatin are bound to 'spacer' DNA regions of nucleosomes [2] and also contact the histone octamer [6–8], the N-terminal half of histone H1 participating in these contacts [9].

At present, there are only indirect data concerning arrangement of H1 molecules relative to each other. However, histone H1 molecules are closely spaced in chromatin and can be crosslinked with bifunctional reagents into oligomeric chains [11]. Treatment of chromatin with MMB leads to the crosslinking of H1 molecules into discrete oligomers, consisting of, e.g., 12, 24, 36 molecules of histone H1 [12].

Using a similar reagent, dimethyl-3,3′-dithiobispropionimidate, H1 dimers were isolated in [8].

Here, we have used limited chymotryptic digestion of H1 dimers to determine what parts of the neighbouring H1 molecules are close enough to be crosslinked in compact nuclear chromatin.

Abbreviations: MMB, methyl-4-mercaptobutyrimidate; SDS, sodium dodecylsulphate; Tris, tris-(hydroxymethyl)aminomethane; DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); PMSF, phenylmethylsulphonyl fluoride

2. Methods

Isolation of calf thymus nuclei, crosslinking of the nuclei with MMB and acid extraction of histone oligomers were performed as in [12], with the following modifications. Concentrations of MMB was increased to 0.3 mg/ml, and the protein was precipitated with 15% trichloroacetic acid prior to gel-filtration. Gel-filtration on a Sephadex G-200 column was done in the presence of 2 M guanidine–HCl. Fractions were collected, precipitated with acetone (14 vol.) and then dried.

H1 histone or H1 dimers (0.5 mg), obtained after gel-filtration (see below), were dissolved in 0.1 ml 50 mM Tris–HCl buffer (pH 8), and digested with α-chymotrypsin (Boehringer) at enzyme/substrate weight ratio 1:500 for 1 min at 37°C. The reaction stopped with 1 mM PMSF (Pierce), protein products were precipitated with 20% trichloroacetic acid, washed with acetone and then dried.

Prior to electrophoresis, the protein obtained was dissolved in the sample application buffer [13] with 5 M urea added and without β-mercaptoethanol, and incubated at 100°C for 15 min. To cleave S–S bridges, the samples were treated similarly but with β-mercaptoethanol added.

The N- and C-terminal chymotryptic fragments of histone H1 comprising amino acid residues 1–106 and 107–213 [14] were purified as in [15].

Electrophoresis in 18% SDS–polyacrylamide gel was carried out in 0.1 × 15 × 15 cm slabs in the buffer system [13], but without a stacking gel. Diagonal gel-electrophoresis was performed in the same system.

After separation in the first dimension (60 V, 16 h), appropriate strips were cut out of the gel and stored in 96% ethanol. To achieve reduction of S–S

bridges in crosslinks before separation in the second dimension, the gel strip was soaked in 50 mM Tris–HCl buffer (pH 8.0), 0.5% SDS, 5% β-mercaptoethanol for 1 h at 25°C. The strip was next immersed in 50 mM Tris–HCl (pH 8.0), 0.5% SDS, 20% ethanol for 2–3 min and put on the top of vertical slab gel for electrophoresis in the second dimension (60 V, 16 h). Gels were then fixed with 0.5% glutaraldehyde [9], stained with Coomassie brilliant blue (Serva), and diffusion destained.

Quantitation of SH-groups in H1 dimers was done with DTNB (BDH) as in [16], using $NaBH_4$ as reducing agent.

3. Results and discussion

3.1. *Isolation and properties of H1 histone dimer*

Chemical crosslinking of histones in nuclei produces dimers of H1 histone [8] as well as longer H1 oligomers [7,11,12]. Crosslinking of calf thymus nuclei, extraction and gel-filtration of the nuclear protein in the presence of guanidine–HCl resulted in 4 protein peaks (fig.1).

Peak 1 contained polymers of histone H1 and was analysed in [12]. During electrophoresis, major protein material from peak 2 migrated slower than histone H1 (fig.2, lane 2) but significantly faster than the H1 polymeric material (not shown).

Cleavage of S–S bonds in crosslinks with β-mercaptoethanol caused almost complete disappearance of major slowly migrating band (fig.2, lane 1). The

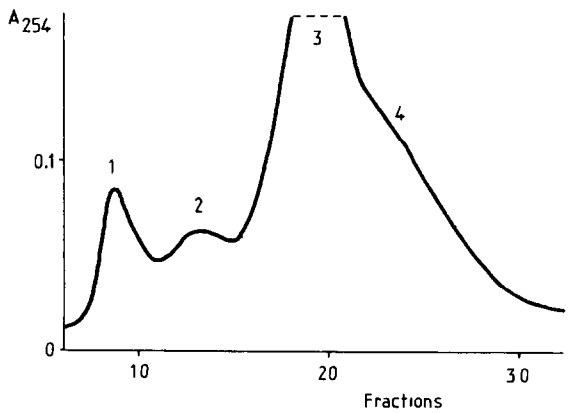

Fig.1. Sephadex G-200 gel-filtration of the MMB-crosslinked histone H1 oligomers: (1) H1 polymer; (2) H1 dimer; (3) H1 monomer; (4) core histones.

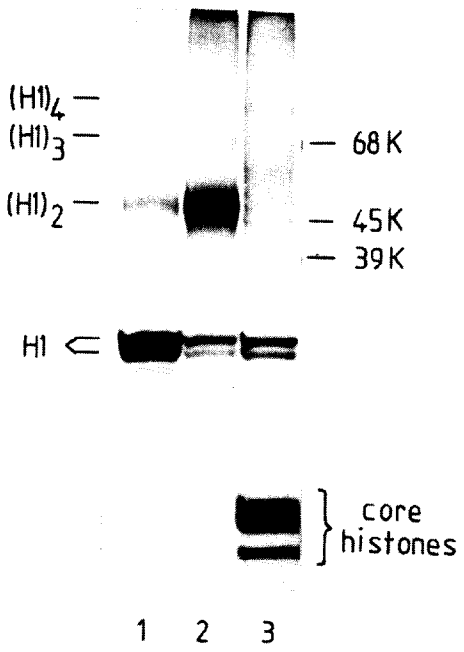

Fig.2. 18% SDS–polyacrylamide gel electrophoresis of the MMB-crosslinked H1 histone dimer: (1) H1 dimer split with β-mercaptoethanol; (2) initial H1 dimer; (3) calf thymus total acid-soluble protein [12]; M_r standards were the same as in fig.6.

intensity of the H1 histone band was simultaneously increased and only minor new bands have been observed beneath this band. The new bands are likely to represent trace amounts of proteolysis products. Since chymotryptic fragments of the slowly migrating fraction comigrate with those of H1 histone (see section 3.3), this fraction does represent an MMB-mediated oligomer of H1 histone. An electrophoretically determined app. M_r of the peak 2 major protein was found to be 49 000. This value slightly exceeds the doubled M_r of histone H1 (42 000) and is somewhat less than that calculated from electrophoretic mobility in the same gel (56 000).

Apart from major band (and H1 histone band) there were also two minor bands (fig.2, lane 2) corresponding to app. M_r of 67 000 and 80 000. Two-dimensional electrophoresis (not shown) confirmed that these bands were converted into the H1 band after cleavage of S–S bonds, and thus also represented H1 oligomers. The actual absence of reduction products distinct from histone H1, and also M_r values, suggest that the 3 oligomeric bands correspond in order of their decreasing electrophoretic mobilities

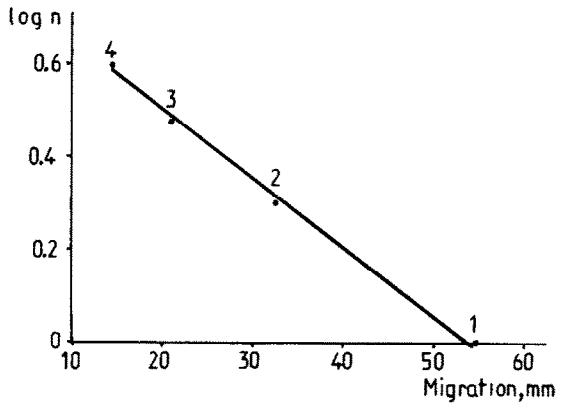

Fig.3. Relationship between migration distances of different H1 oligomers in gels and logarithms of their M_r values (see text).

to dimer, trimer and tetramer of histone H1, respectively. This is supported by the linear relationship between migration distances of the fractions and logarithms of their expected relative M_r (fig.3). The major protein component of peak 2 can thus be unambiguously assigned to H1 dimer.

Peaks 3 and 4 in fig.1 contained H1 monomers and core histones, respectively (not shown).

The yield of histone H1 dimer was ~10% of that for mono-H1.

The number of MMB SH groups per H1 molecule derived from peak 2 proteins was found to be ~0.8. The correct value should be somewhat higher due to admixture of H1 monomers in the peak 2 fraction (fig.2, lane 2). This suggests that the H1 dimers contain mostly only one MMB–MMB bond.

3.2. *Electrophoresis of the chymotryptic fragments of histone H1 in SDS–polyacrylamide gels*

Limited chymotryptic treatment induces splitting of the histone H1 molecule into two characteristic N- and C-terminal fragments comprising amino acid residues 1–106 and 107–213, respectively [14]. The fragments will be hereafter also referred to as N- and C-fragment or simply N and C.

Fig.4 (lane 2) shows the separation of a chymotryptic digest of histone H1 in SDS–polyacrylamide gel. The band were assigned using purified N- and C-fragments of histone H1 (the C-fragment shown in fig.4, lane 3). Like intact H1 molecules, the C-fragment migrated in a gel as two separate bands (C_a and C_b, fig.4) whereas the N-fragment migrated as a single band.

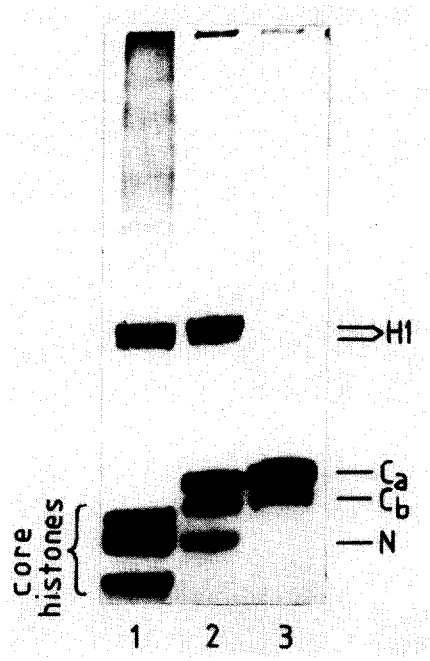

Fig.4. 18% SDS–polyacrylamide gel electrophoresis of the limited chymotryptic digest of H1 histone: (1) calf thymus total acid-soluble protein; (2) chymotryptic digest of H1 histone; (3) homogenous C-terminal fragment of H1 [15].

3.3. *Chymotryptic digestion of H1 dimers*

Histone H1 dimers can be imagined to contain several different types of the intermolecular crosslinks (fig.5, I–III). IV represents possible intramolecular MMB-bonds. To determine the character of crosslinking in the H1 dimeric fraction from nuclei, we used diagonal gel-electrophoresis (fig.6). The protein spots located outside the diagonal should obviously correspond to species kept together due to MMB-crosslinks.

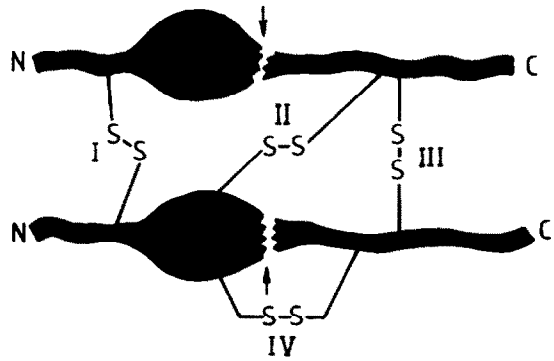

Fig.5. Schematic representation of the H1 histone dimer: (I–IV) possible types of MMB-crosslinks in the dimer. Arrows indicate the chymotrypsin cleavage sites.

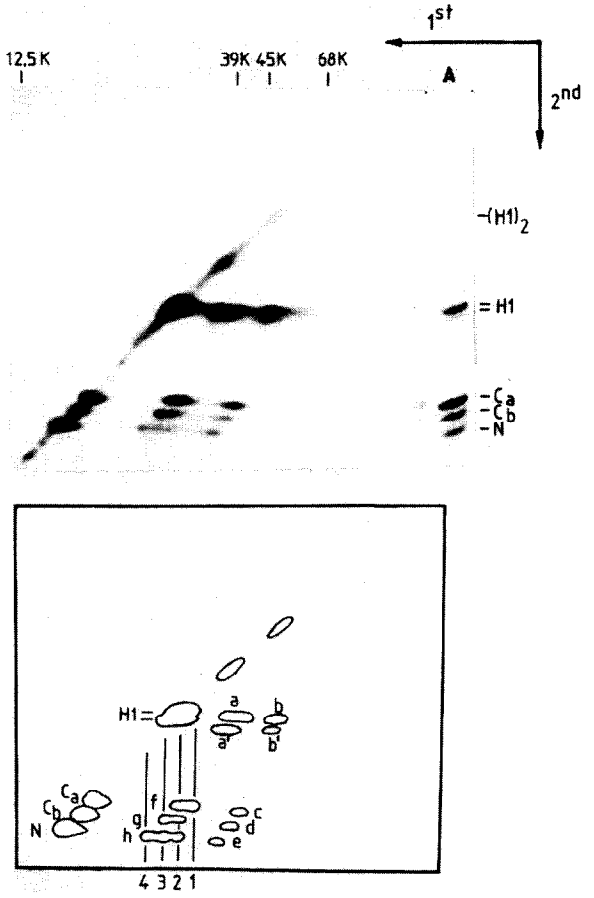

Fig.6. Diagonal gel-electrophoresis of the H1 dimer chymotryptic digest. M_r standards: 68 000, bovine serum albumin; 45 000, ovalbumin; 39 000, rabbit muscle aldolase monomer; 12 500, cytochrome *c*. (A) Standard H1 histone digest. For further details see the text.

The protein of extra-diagonal spots b and b′ (fig.6) migrated as H1 dimer in the first dimension and H1 monomer in the second and thus was crosslinked H1 dimer. Absence of the spots corresponding to the H1 fragments beneath the spots b and b′ demonstrates that the cleavage of any H1 molecule in the dimer is enough to convert the latter into lower M_r species. On the other hand, prolonged digestion of crosslinked dimers with chymotrypsin led to complete splitting of all the H1 molecules within the dimers. These findings enable us to deduce the absence of type IV intramolecular crosslinks (fig.5) in isolated dimers.

The electrophoretic mobility of the precursor of spots a, a′, c, d and e in the first dimension corresponded to an aggregate consisting of one H1 molecule attached to one of the H1 chymotryptic fragments (fig.6). The spots a and a′ represented intact H1 histone, and the spots c, d and e apparently corresponded to chymotryptic fragments of the complexes H1–C_a, H1–C_b and H1–N, respectively.

The spots f, g and h (fig.6) seems to be a result of reduction of crosslinked in pairs H1 chymotryptic fragments.

In as much as the C_b and N-fragments were not found beneath the spot f on the vertical line 1 and the electrophoretic mobility of this part of the spot f precursor in the first dimension was somewhat less than that of H1, the right part of the spot f represents the C-fragments from C_a–C_a crosslinked dimers. This dimer has the least mobility of all possible pairs of the H1 chymotryptic fragments.

The left part of the spot h (vertical line 4) can be similarly concluded to contain the N-fragments from split N–N dimers, which should have the highest mobility.

Both C- and N-fragments were located on the vertical lines 2 and 3. Therefore they originated from crosslinked molecules, which nearly comigrated with histone H1 in the first dimension and contained both the N- and C-fragments of histone H1. These properties are in accord with those expected for crosslinked C_a–N and C_b–N species.

It is difficult to locate precisely the spots corresponding to C_a–C_b and C_b–C_b crosslinked species because of non-sufficient separation of this species in the first dimension. However, these crosslinks are also very likely.

These data provide unambiguously evidence that the mutual arrangement of histone H1 molecules in chromatin of intact nuclei is such that the H1 halves can be crosslinked in all possible combinations, i.e., C–C (at least C_a–C_a), C–N and N–N.

If the H1 arrangement in extended nucleosomal chains is head-to-tail as supposed in [10], the N–C crosslinks should be a direct consequence of this arrangement. In this case C–C and N–N pairs could be due to the package of nucleosomes in the superstructure. This supposition can be tested by comparison of crosslinking patterns of histone H1 in nuclei and extended chromatin. This work is now under way.

Acknowledgements

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